DSPE).--

-- 18. The composition of claim 10, wherein said antineoplastic phospholipid is OPP, and said antineoplastic antiestrogen is tamoxiphen. –

REMARKS

Claims 9-18 are in the application.

Also enclosed herewith is a comparison copy of the substitute disclosure showing the changes. No new matter was added.

Favorable consideration of the application, as amended, is respectfully urged.

Respectfully submitted

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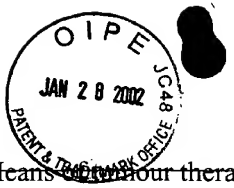
It is hereby certified that this is being mailed on January 8, 2002.

Francene Sawyer



~~Means of tumour therapy~~] **0107-032**

~~[Description]~~ Tumor Treating Composition

~~[The invention in question]~~ **Field of the invention**

The present invention relates to a pharmaceutical ~~[agent on the basis of acombination of anti-oestrogen]~~ **composition of an antiestrogen**, alkylphospholipids and phospholipids, its manufacture and use.

~~[Fields of application of the invention are medicine and the pharmaceutical industry. In medicamentous tumour]~~ **Background**

In tumor drug therapy, optimal treatment is repeatedly inhibited by the occurrence of resistance against the ~~[pharmacon]~~ **drug** and by toxic side ~~[-]~~effects. ~~[A part]~~ **Some** of these undesired effects can be ~~[cancelled]~~ **eliminated** or ~~[soothed]~~ **reduced** by encapsulation of the ~~[medicaments]~~ **drugs** in liposomes (D. D. Lasic and D. Papahadjopoulos, Medical Applications of Liposomes, Elsevier, 1998). Liposomal anthracyclins have ~~[reached the stage of extended]~~ **been employed in numerous** clinical ~~[application]~~ **applications**. Specific benefits result if phospholipids with an inherent ~~[anti-tumour]~~

antitumor effect are used to form the liposomes, e.g. alkyl phospholipids (Arndt et al. Drugs of Today 1998, 34, 83-96).

Alkyl phospholipids are ~~[a]~~ **relatively** new type of ~~[compound,]~~ **compounds,** the ~~[effect]~~ **effects** of which ~~[against tumour]~~ **on tumor** growth is achieved by **their** effects on the cell membrane (Alkylphosphocholines: An update, Drugs of Today, Vol. 34, Suppl. F, 1998). Under certain conditions, alkylphospholipids ~~[result in supra-molecular]~~ **have supramolecular** structures, ~~[inter alia]~~ **such as** liposomes, with more ~~[favourable]~~ **favorable** properties ~~[compared with]~~ **than** the monomeric or micellar ~~[organized compound (DE 41 32 345 A1, DE 44 08 011]~~ **compound (German patents Nos. 4,132,345 A1; and 4,408,011** C1). Further substances ~~[with anti-neoplastic]~~ **having an antineoplastic** effect can **also** be included in these liposomes ~~[with an inherent anti-tumour]~~ **that have an antitumor** effect (Arndt et al., Breast Cancer Res. Treatm. 43 (1997) 237-246, ~~[DE 44 08 011 C1).~~ ~~]~~**German patent No. 4,408,011 C1).**

~~[Mamma carcinomas, the most frequent tumour in women,]~~ **Breast cancer is the most frequently occurring tumor in women. It** can be influenced in ~~[about 75% of the]~~ **most** cases by endocrine measures**, as can also other cancers such as of the prostate, uterus, brain, and thyroid cancers**. Competitive hormone therapy ~~[by means of Tamoxifen]~~ **with tamoxifen** is of particular importance in this context; in it, the endogenous hormones are ~~[antagonised]~~ **antagonized** at the receptor. Treatment with ~~[Tamoxifen,]~~ **tamoxifen,** which ~~[is low in]~~ **has only a few** side-effects, is however limited by development of resistance against the ~~[pharmacon]~~ **drug**. The causes of ~~[the]~~ **this** resistance ~~[are, inter alia,]~~ **include** alterations of the ligand and its binding to the ~~[oestrogen]~~ **estrogen** receptor (ER), loss or alteration of the ER, alterations of transcription factors or the ER-associated protein or blockage through anti-~~[oestrogen]~~ **estrogen** binding proteins (Katzenellenbogen et al., Breast Cancer Res. Treat. 44 (1997) 23-38; Osborne, New Engl. J. Med. 339 (1998) 1609-18; ~~[US005904930A).]~~ **US patent No. 5,904,930).**

~~[The objective of the invention is the creation of a medication formulation on the basis of anti-oestrogen, alkylphospholipid and phospholipids.]~~ **Brief description of the invention**

It is an object of the present invention to provide an antineoplastic alkylphospholipid in combination with an estrogen in a lipid vesicle (i.e. a liposome) which is effective in ~~[anti-oestrogen]~~ **antiestrogen** resistant ~~[tumours]~~ **tumors** and which ~~[minimises]~~ **minimizes** or prevents the development of resistance.

The ~~[invention is characterised by the primary claims, the sub-claims being preferred variants]~~ **present invention is a pharmaceutical composition which comprises a combination of an antineoplastic alkyl-phospholipid, a water -or lipid-soluble antiestrogen in a lipid vesicle, and a phospholipid, such as phosphatidylcholine, that has no antineoplastic properties. The composition can optionally also include a cholesterol or other sterol, a lipid with a positive or negative charge, and a polyethylene glycol-modified PEG lipid and/or pharmaceutical carriers and/or excipients.**

~~[.]~~ **Brief description of the drawing**

The sole figure of this application shows the cytotoxic effect of tamoxifen liposomes on breast cancer cells.

Detailed description

The essential feature of the invention is ~~[the combination of alkylphospholipid with an anti-neoplastic effect and an anti-oestrogen]~~ **a composition which contains an antiineoplastic alkylphospholipid, and an antineoplastic antiestrogen** in a lipid vesicle. A ~~[preferred]~~ **suitable** example **of these ingredients** is octadecyl-(N,N-dimethylpiperidin-4-yl)-phosphate (OPP), ~~[Tamoxifen (Tam) in phosphocholine (PC) vesicles.]~~ **hexadecylphosphocholine, erucylphosphocholine, octadecylphosphoethanolamine, and hexadecylphosphoserine.**

~~[In detail, the agent according to the invention is characterised by the following composition:~~

~~]~~**More particularly, the composition of the present invention contains (a)** an alkylphospholipid **with antineoplastic effect, (b) a water -or lipid-soluble antiestrogen in a lipid vesicle, and (c) an antineoplastically inert phospholipid, and optionally (d) one or more of**~~[(with anti-neoplastic effectivity)~~

~~- a water or lipid-soluble anti-oestrogen with anti-neoplastic effectivity~~

~~- an anti-neoplastically inert phospholipid~~

~~- if need be,]~~ cholesterol or any other suitable sterol, **and**~~[~~

~~- if need be,]~~ a lipid with positive or negative surface charge, **and**~~[~~

~~- if need be,]~~ a polyethylene glycol modified lipid (PEG lipid), **and further actives as well as a pharmaceutically conventional carrier and/or excipient.**

As used herein, "antineoplastically inert" means a compound that has no antineoplastic properties.

The alkylphospholipids of the present composition suitably has the formula **R-Y-P-X** **(1)**

wherein

R is a C_{12-22}

~~- if need be, further active agents and pharmaceutically customary carrier and ancillary materials.~~

~~Alkylphospholipids with an anti-tumour effect of general structure I are used as phospholipid analogs.~~

~~Structure I. R-Y-P-X~~

~~This formula contains the following meanings:~~

~~R: an~~ alkyl, alkenyl or alkinyl residue ~~[with 12 to 22 C atoms]~~;

~~[Y:]~~ **Y is** oxygen, ~~[sulphur]~~ **sulfur** or **a** CH_2 **residue;**

P ~~[:]~~ **is a** phosphate group (PO_2); **and**

X ~~[:]~~ **is** a choline ~~[or]~~, modified choline ~~[rest]~~ **residue** or serine, ethanolamine, glycerine ~~[groups or synthetic modifications of these groups such as the piperidine-4-yl group]~~ **group, or a synthetic modification of the foregoing groups.**

~~[Preferred compounds are]~~ **Suitable examples of X include** hexadecylphosphocholine, octadecylphosphocholine, erucyl- phosphocholine,

octadecyl-[2-(N-methylpiperidinio)ethyl]-phosphate, octadecylphospho-ethanolamine and hexadecylphosphoserine. **A suitable example of a synthetic modification is the piperidine-4-yl group.**

A ~~[The]~~ water or lipid-soluble ~~[anti-oestrogen]~~ **antiestrogen** associated with the phospholipid analogs ~~[is represented by Tamoxifen, Droloxifene, Toremifene, Idoxifene, Raloxifene, Miproxifene-Phospat]~~ **of Formula (I) is suitably tamoxifen, droloxifene, toremifene, idoxifene, raloxifene, miproxifene-phospate** (TAT-59), ICI 1643,384, ICI 182,780 and the main metabolites of ~~[Tamoxifen,]~~ **tamoxifen, namely** 4-hydroxytamoxifen and N-~~[desmethyltamoxifen.]~~**desmethyl-tamoxifen.**

~~[Phospholipids]~~ **Antineoplastically inert phospholipids** without their own ~~[anti-neoplastic]~~ **antineoplastic** effect are **generally** lipids from natural sources or of synthetic origin such as **are** customarily used for liposome production, ~~[e.g.]~~ **for example** phosphatidylcholine.

~~[Preferably,]~~ **Suitably** polyethylene glycol modified phosphatidylethanolamine in the molecular weight range of 1000 - 6000 Dalton is used as a PEG lipid. ~~[Inter alia, 1,2-Distearoyl]~~ **For example, suitable compounds include** **1,2-distearoyl**-s,n-glycero-3-phosphoethanolamine-N-polyethylenglycol, MG2700; (PEG_{2000}DSPE) and 1,2-~~[Dipalmitoyl]~~ **dipalmitoyl**-sn-glycero-3-phosphoethanolamine-N-polyethylenglycol, MG5750 (PEG_{5000}DPPE) ~~[are suited. The use of compounds]~~**. Compounds** which are simultaneously a PEG lipid and an anti-neoplastically effective phospholipid analog ~~[is]~~**, are** also ~~[beneficial, for example]~~ **useful, such as** hexadecylphosphoethanolamine-N-~~[polyethylenglycol]~~ **polyethyleneglycol**.

According to the invention, **suitably** an anti-neoplastically inert lipid of a natural or synthetic origin is ~~[preferably]~~ used as a base lipid for the membrane formation, such as phosphocholine, serine, ethanolamine, glycerol or other similar lipids, with the ratio of lipid to ~~[anti-oestrogen]~~ **antiestrogen** being **from** 0 ~~[-]~~**to** 10 : 1 (mass ratio m/m).

[Preferably] **Suitably**, cholesterol or another suitable sterol such as sitosterol is [contained,] **used** with the sterol being in a mol ratio of **from** 0 [-]**to** 1 : 1 to the alkylphospholipid. [

]The liposomal form [preferably comprises] **is suitably a** single-layered or [multi-layered vesicles] **multilayered vesicle** or the liposomes are available as ["] **a** reverse evaporation [vesicles"] **vesicle**.

The effect of the agent [of overcoming] **to overcome** resistance according to the **present** invention can be [proven] **shown** both *in vitro* and *in vivo*. The [means of tumour therapy according to the]**composition of the present** invention is pharmaceutically stable, physiologically outstandingly tolerable**,** and **is** particularly [suitable] **suited** for intravenous application. Undesired metabolism of the [anti-oestrogens] **antiestrogens** is avoided or reduced, **and** improved resorption and distribution of the [pharmacon] **drug** is achieved. [Anti-oestrogens] **Antiestrogens that are** difficult to dissolve in water can [well] be **easily** applied in a liposomal form. The [means] **composition of the present invention** is therefore [outstandingly] **very well** suited for application in [tumour] **tumor** therapy.

The invention is ~~[explained by]~~ **further illustrated through** the following examples~~[:]~~**.**

Example 1:

4.62 mg octadecyl-(1,1-dimethyl-piperidino-4-yl)-phosphate (OPP; 10 µmol), 0.387 mg Z-4-hydroxy-~~[Tamoxifen]~~ **tamoxifen** (HO-Tam, 1 µmol), 1.55 mg cholesterol (4 µmol), and 1.1 mg dicetylphosphate (DCP; 2 µmol) are completely dissolved in 25 ~~[ml]~~ **mℓ** chloroform/methanol (7/3; v/v) and the solvent **is** then completely evaporated on a rotation evaporator. The finely distributed lipid film ~~[gained is re-suspended]~~ **that is obtained is resuspended** with 1 ~~[ml]~~ **mℓ** of phosphate-buffered salt solution (PBS, pH 7.4) and intensively moved for at least 3 hours at room temperature on a vibration machine following addition of some glass pearls. The **resulting** suspension of ~~[multi-layered]~~ **multilayered** vesicles (MLV) ~~[obtained]~~ is then repeatedly extruded through polycarbonate filters~~[,]~~ **of a** pore diameter **of** 100 nm, with a LiposoFast basic system ~~[(]~~**(sold by** Avestin, Inc. Ottawa, Canada) until vesicles with an average diameter around 100 nm with a unimodal distribution of sizes and a polydispersity index of less than 0.2 ~~[(]~~**(as determined by** Dynamic Light Scatter Measurement, DLS) are obtained.

The content of OPP, HO-Tam, CH and DCP is checked by ~~[means of]~~ HPTLC. ~~[Above]~~ **Over** 85 % of the original amount is retained. The composition of the liposomes is unchanged compared with the original composition (deviation < 5%). These HO-Tam liposomes are ~~[preferably]~~ **most suitably** used for *in vitro* ~~[examinations]~~ **tests**.

Example 2:

~~[.]~~ 36 mg OPP, 72 mg ~~[Tamoxifen]~~ **tamoxifen** citrate (Tam), 144 mg phosphatidylcholin (PC) and 8.5 mg DCP are completely dissolved in 100 ~~[ml]~~ **m**ℓ chloroform/methanol (7/3; v/v) and the solvent then completely evaporated on a rotation evaporator. The **resulting** finely distributed lipid film ~~[gained re-suspended]~~ **is resuspended** with 12 ~~[ml of]~~ **m**ℓ citric acid/phosphate buffer (pH 6.08), and intensively moved for at least 3 hours at room temperature on a vibration machine following addition of some glass pearls. An MLV suspension is obtained, which is heterogeneous **and** in its size ~~[composition with]~~ **distribution has** vesicle diameters of between 100 and 5000 nm.

These Tam liposomes are ~~[preferably]~~ **most suitably** used for *in vitro* ~~[examinations]~~ **tests** and as initial liposomes for vesicles of a defined size.

Example 3

36 mg OPP, 72 mg ~~[Tamoxifen]~~ **tamoxifen** citrate (Tam), 144 mg phosphatidylcholine (PC) and 8.5 mg DCP and ~~[additionally]~~ 9.7 mg N-(O-methyl-polyethylenglycyl)-1,2-distearyl-s,n-glycero-3-phosphoethanolamine ($PEG_{2000}DSPE$) are completely dissolved in 100 ~~[ml]~~ **mℓ** chloroform/methanol (7/3; v/v) and the solvent then completely evaporated on a rotation evaporator. The **resulting** finely distributed lipid film ~~[gained is re-suspended]~~ **is resuspended** with 12 ~~[ml]~~ **mℓ** of citric acid/phosphate buffer (pH 6.08) and intensively moved for at least 3 hours at room temperature on a vibration machine following addition of some glass pearls. An MLV suspension is obtained, which is heterogeneous in its size ~~[composition with]~~ **distribution has** vesicle diameters of between 100 and 5000 nm. These Tam liposomes are ~~[preferably]~~ **most suitably** used for *in vitro* ~~[examinations]~~ **tests** and as initial liposomes for vesicles of a defined composition.

Example 4:

Tam MLV's from ~~[example]~~ **Example** 2 are repeatedly extruded through polycarbonate filters, pore diameter 200 nm, with a LiposoFast basic system (Avestin, Inc. Ottawa, Canada) until a unimodal size distribution around 180 nm is achieved with a poly-dispersity index of less than 0.35 (Dynamic Light Scatter Measurement, DLS).

The content of OPP, Tam, CH and DCP is checked by ~~[means of]~~ HPTLC. A liposome suspension containing about 75 % of used Tam and 98 % of OPP is obtained. In addition, the composition of the liposomes is unchanged compared ~~[with]~~ **to** the original composition (deviation < 5%). These Tam liposomes are ~~[preferably]~~ **most suitably** used for *in vivo* ~~[examinations]~~ **tests**.

Example 5:

Peg-Tam MLV's from ~~[example]~~ **Example** 3 are repeatedly extruded through polycarbonate filters, pore diameter 200 nm, with a LiposoFast basic system (Avestin, Inc. Ottawa, Canada) until a unimodal size distribution around

185 nm is achieved with a poly-dispersity index of less than 0.33 (Dynamic Light Scatter Measurement, DLS). ~~[~~

~~]~~The content of OPP, Tam, DCP und Peg_{2000}DSPE is checked ~~[by means of]~~ **with** HPTLC. A liposome suspension containing about 75 % of used Tam and 98 % of OPP is obtained. In addition, the composition of the liposomes is unchanged compared with the original composition (deviation < 5%). The Peg-Tam liposomes are ~~[preferably]~~ **most suitably** used for *in vivo* ~~[examinations]~~ **tests**.

Example 6:

HO-Tam liposomes from ~~[example]~~ **Example** 1 are diluted with **an** RPMI medium with 10% ~~[foetal]~~ **fetal** calves' serum (without added indicator, with ~~[adriamycin/streptomycin) in such a way]~~ **adriamycin/ streptomycin) so** that a concentration of 200 ~~[nmol/ml]~~ **nmol/mℓ** of OPP is reached, then ~~[being]~~ further serially diluted down to 0.78 ~~[nmol/ml]~~ **nmol/mℓ**. The concentration of HO-Tam active agent is then accordingly **from** 20 ~~[nmol/ml]~~ **nmol/mℓ** to 0.08 ~~[nmol/ml.]~~ **nmol/mℓ.**

~~[The breast]~~ **Breast** cancer cells MCF7, which are sensitive ~~[towards Tamoxifen]~~ **tamoxifen**, and MCF7-R, which are resistant to ~~[anti-oestrogen]~~ **antiestrogen**, are seeded into 96-well plates with a density of $2x10^4$ cells/well and incubated on the following day with HO-Tam liposomes, control liposomes of the composition of the HO-Tam liposomes, but without HO-Tam, HO-Tam~~[,]~~ dissolved in DMSO and DMSO of the same amount as needed to dissolve the HO-Tam, for three days. ~~[After this, the]~~ **The** supernatants are **then** removed, the cells washed with PBS and then the cell growth inhibition determined with the MTT assay. ~~[For this, the]~~ **The** cells are incubated **for this** with 200 ~~[µl]~~µℓ MTT solution (4,6-dimethylthiozol-2-yl-2,5-diphenyl-tetrazolium; 0.5 ~~[mg/ml)]~~ **mg/mℓ)** for 4 hours at 37°C, 170 ~~[µl]~~µℓ of the supernatant **is** carefully removed and the precipitated formasan crystals completely dissolved with a 70% ~~[Isopropyl]~~ **isopropyl** alcohol solution by intensive pipetting and shaking. After this, the 96-well plates are photospectroscopically measured at 540 nm and the growth inhibition calculated in comparison to the growth of untreated cells. A growth inhibition as portrayed in Figure 1 is obtained.

Example 7

[.] 1 X 10^5 cells/mℓ were incubated with the corresponding liposomes (L), HO-TAM/DMSO and with DMSO for 3 days. The living cells were determined with the MTT assay. The concentration of active agent necessary to inhibit the cell growth by 50% (IC_{50}) is stated.

Tam liposomes according to Example 4 are used for the *in vivo* **treatment** test. As a [tumour] **tumor** model, breast cancer 3366/Tam is transplanted onto female NMRI nude mice and the treatment started when the [tumour] **tumor** is palpable. The animals are given one dose of liposomes with 50 mg/kg Tam (and correspondingly 25 mg/kg OPP) twice a day for 4 weeks. As controls, liposomes containing no Tam are administered, in addition one group being treated with free Tam. The [tumour] **tumor** growth in relation to the control group (physiological salt solution) is determined and portrayed as a percentage T/C [figure in Table 1.], **as shown in Fig. 1 and in Table 1. The example of Fig. 1 shows that 1 x 10^5 cells/ml were incubated with the corresponding liposomes (L), HO-TAM/DMSO and with DMSO for 3 days. The living cells were determined with the MTT assay. The concentration of active agent**

necessary to inhibit the cell growth by 50% (IC_{50}) is represented. The asterisk * means that the result is significantly different from HO-TAM; and a plus sign + means that the r4esult is: significantly different from MCF7(R-).

Table 1[

]:

Therapeutic effectivity of ~~[Tamoxifen]~~ **tamoxifen** liposomes compared with the resistant breast cancer ~~[tumour]~~ **tumor** 3366/Tam

Group	Substance	Dose, Tam/Lipid	Alteration of body weight	**T/C**
		mg/kg/injection	% (day 29/51)	%
A	Solvent		3	
B	~~[Tamoxifen]~~ **tamoxifen**	50/0	-5	91
C	~~[Tamoxifen]~~ **tamoxifen** liposomes	50/25	-5	63*
D	~~[Control]~~ **control** liposomes	0/25	-4	88

~~[* Significantly different from Tamoxifen and the solvent control ($p \leq 0.05$)]~~

~~[Patent claims]~~* **Significantly different from Tamoxifen and the solvent control ($p < 0.05$)**